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PRINCIPAL INVESTIGATOR: Keith D. White, Ph.D.

CONTRACTING ORGANIZATION:University of Florida, Gainesville, FL 32611

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14. ABSTRACT
The overarching objective of this study is to characterize abnormalities of vestibulo-ocular reflexes (VOR) in Autism Spectrum Disorder (ASD). Specific Aim 1: Characterize horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades. Specific Aim 2: Characterize horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls. Specific Aim 3: Characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback. The present report covers the second year following award initiation. Complete research data have been obtained from 16 children with ASD and 36 typically developing children.

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Vestibulo-ocular reflex, autism

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INTRODUCTION

The overarching objective of this study is to characterize abnormalities of vestibulo-ocular reflexes (VOR) in Autism Spectrum Disorder (ASD). Specific Aim 1: Characterize horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades. Specific Aim 2: Characterize horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls. Specific Aim 3: Characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback. Because neither of these aspects of the VOR have been described previously in ASD, Aim 3 is exploratory.

The present report covers the third year following award initiation on 15 May 2010. Limited data were obtained during Year 1 due to equipment issues that were not solved until Year 1 Month 12. During the second year, it was possible to obtain complete research data from one child with ASD and 18 typically developing children. During the third year, complete research data were obtained from 15 additional children with ASD and from 17 additional typically developing childen.

As of May 14th, 2013, recruitment efforts by the lab have generated contacts regarding 34 ASD children and 36 typically developing children. Out of this number of potential recruits, 24 children with ASD and 36 typically developing children have actually come to the lab and their parents have given informed consent for data collection preparatory to research. Of those whose parent/caregiver gave informed consent, for 24 of the children with ASD and all 36 typically developing children, sufficient data could be obtained to determine that they could be enrolled in the neuropsychological testing phase of the study. Out of these 36 typically developing children, two participated in vestibulo-ocular testing protocol used to “fine-tune” the exact experimental procedures such that their data are not strictly comparable to data obtained in the formal protocol. Also, out of those 24 potentially enrolled ASD children, one did not qualify for enrollment in the vestibulo-ocular testing phase of the study because their Autism Diagnostic Observation Schedule (ADOS) score did not confirm the suspected diagnosis of ASD. Out of the 34 typically developing participants enrolled in the formal research protocol, 25 have provided all of the data to be collected under the protocol. Two typically developing children were found to have abnormal oculomotor profiles, and were thus excluded as control subjects for vestibulo-ocular reflex testing. The other 7 typically developing children who have partial data collected appear to be lost to follow-up due to lack of parental responses to reminders.

Out of the 23 ASD participants enrolled in the vestibulo-ocular testing phase of the protocol, 16 have provided all data to be collected under the protocol. Of the 7 ASD children having partial data collected: 1 appears to be lost to follow-up (lack of parental responses); 2 of these ASD children with partial data collected nearly completed all conditions but were excused from completing the study due to challenged capacity to follow instructions; 3 of these ASD children were unable to complete any further VOR tests and were excused from completing the study due to challenged capacity to follow instructions; and 1 has been scheduled for testing on 28 May 2013 (during no-cost extension of the project).

Overall, the VOR data obtained from typically developing children seem entirely consistent with norms from the literature, as further described below. This correspondence leads us to have confidence that the equipment is operating properly and that our procedures are representative.

BODY

Statement of Work: Abnormal Vestibulo-Ocular Reflexes in Autism: A Potential Endophenotype

Task 1. Activities preparatory to research (year 1, months 1 - 6)

Subtask 1a. Submit protocol for human research participation to UF Institutional Review Board.

Milestone #1: Human research participation approval by UF Institutional Review Board was granted in April 2010, renewed in April 2011 and in April 2012, and granted by ARO Human Research Protections Office (HRPO Log No. A-16019). Minor changes to the protocol, such as creation of a phone screening form for recruitment, have been approved by the UF IRB and HRPO on several occasions. Milestone #1 is on track.

Subtask 1b. Research assistants trained in administration of Autism Diagnostic Observation Schedule (ADOS), certification required, and other testing administration.

Milestone #2: Neuropsychological and vestibulo-ocular reflex tests ready to be administered by research assistants. Neuropsychological testing kits and materials have been acquired and renewed as used up. Tana Bleser, Graduate Research Assistant, and Jill Weish, Graduate Research Assistant, completed ADOS training. Milestone #2 is on track.

Subtask 1c. Submit protocol for recruitment of research participants to Alachua County School District, to local therapy centers having ASD clients, and to UF Center for Autism and Related Disorders (CARD).

Milestone #3: Permission granted to recruit on premises (schools, therapy centers) or via a contacts database (CARD). Milestone #3 is presently on track. Approximately 2000 recruitment fliers were distributed in public and private schools before the school year ended, and several therapy centers also have fliers. Participants are currently responding to those fliers. We distribute new fliers periodically and visit premises to refresh permissions as needed.

Task 2. Acquire eye tracking apparatus and set it up on site integrated with existing equipment (year 1, months 1 - 9). The existing equipment had to be replaced during year 1 which cuased several months of delays .

Subtask 2a. Establish appropriate levels of infrared illumination for high frame rate eye tracking while maintaining low visibility to the participants. This has been achieved during year 2.

Subtask 2b. Synchronize eye tracking data acquisition and rotary device motion control computers. This has been achieved during year 2.

Subtask 2c. Create data base structures to link eye tracking data and rotary motion data to neuro-psychological results. This work has been achieved during year 2.

Subtask 2d. (year 1, months 1 - 9) Research assistants trained in administration of vestibulo-ocular reflex tests, data entry procedures, and data quality control/quality assurance. This work has been achieved during year 2.

Milestone #4: Equipment and software ready for testing human participants. This milestone has been met.

Task 3. Recruitment and testing of 8 pilot study participants (year 1, months 6 - 12). Recruitment of research participants is from Alachua County public schools, local therapy centers having ASD clients, and the UF Center for Autism and Related Disorders (CARD). All research testing and laboratory work takes place at the University of Florida. Task 3 commenced Year 1 Month 12, having been delayed by equipment issues explained above.

We have had great success in recruiting and testing 36 typically developing children , exceeding the goal for subtask 3b below. We have had more limited success in recruiting 24 ASD children for subtask 3a below.

Subtask 3a. (year 1, months 6 - 12) Recruitment of 4 ASD research participants from Alachua County public schools, local therapy centers, and UF Center for Autism and Related Disorders (CARD). Administration of the following questionnaires to each set of parent(s)/guardian(s): Children's Communication Checklist-2, Repetitive Behavior Scale-Revised, Vineland-II, and the ShortSensory Profile. Questionaire responses are scored and entered into database. Administration of neuropsychological tests to each ASD child: Autism Diagnostic Observation Schedule (ADOS) and Leiter test of non-verbal problem solving. Scoring and validation of neurpsychological tests and entry into database. Administration of vestibulo-ocular reflex tests to each ASD child. Individual-level analysis made of eye movements to insure valid data capture of the reflexes (data quality control). Individual-level audits made of database record integrity (quality assurance). This subtask is currently in progress. . There have been minor changes to the specifc forms of neuro- psychological tests to be administered, as approved by UF IRB and HRPO.

We have completed testing one child with ASD during year 2 and 15 children with ASD during year 3, and have completed testing the 17th child with ASD early in the no-cost extension year.

Subtask 3b. (year 1, months 6 - 12) Recruitment of 4 non-ASD control participants from Alachua County public schools. This subtask will not begin until 10 ASD participants (50% of the target ASD sample size in Subtask 4a) have been recruited, to allow for the selection of controls who are (as group averages) age-and gender-matched to the ASD participants. Administration of the following questionnaires to parents: Children's Communication Checklist-2, Repetitive Behavior Scale-Revised, Vineland-II, and the Short Sensory Profile. Questionaire responses are scored and entered into database. Administration of Leiter test of non-verbal problem solving to each non-ASD (control) child, which is scored and scores entered into the database. Administration of vestibulo-ocular reflex tests to each non-ASD age- and gender-matched control child. Individual-level analysis of eye movements made to insure valid data capture of the reflexes (data quality control). Individual-level audits made of database record integrity (quality assurance). This subtask is currently in progress. There have been minor changes to the specifc forms of neuro- psychological tests to be administered to child participants, as approved by UF IRB and HRPO.

We have completed testing of 18 non-ASD typically developing children during year 2, and an additional 17 typically developing children during year 3.

Subtask 3c. (year 1, month 12) Compare results from 4 non-ASD pilot participants to the literature for comparability. This subtask was accomplished during Year 3 Month 3.

Subtask 3d. (year 1, month 12) Compare results from 4 ASD pilot participants to preliminary findings cited in the proposal for consistency. This subtask was accomplished during Year 3 Month 6.

Subtask 3e. (year 1, month 12) Correct inefficiencies, if found, in test administration procedures or software or data structures. This subtask has in effect been carried out by testing a larger than planned sample of non-ASD children.

Subtask 3f. (year 1, month 12) Prepare abstract of pilot study findings for presentation at a national professional meeting. This subtask was accomplished during Year 3 Month 9.

Milestone #5: Pilot study of 8 participants supports launch of formal research protocol by the end of grant year 1. Milestone #5 has not been met as originally proposed, but was instead met in an alternative fashion during year 2, by testing a much larger sample of non-ASD participants (18) than the 4 originally proposed. Combined with successful testing of 2 children with ASD (although the second ASD child did not complete testing until May 25, a few days into year 3) we determined that no changes will be needed in the protocol. Milestone #5 was completed for ASD participants during year 3.

Task 4. Prepare annual report of grant activities with pilot study results and tentative conclusions from these pilot results (year 1, month 12). Accomplished June 2011, 2012, and 2013.

Subtask 4a. (year 1, months 11 - 12) Annual renewal of human research participation approvals (UF IRB and HRPO). Accomplished April 2011, 2012.

Summary:

Out of the 24 children with ASD recruited, 16 have completed data collection under the protocol by the end of year 3. Out of the 36 typically developing children recruited, 25 have completed data collection under the protocol by the end of year 3.

Representative findings comparing ASD and typically developing (TD) groups: Key differences highlighted in bold font.

Table 1. Summary of Group Mean (SD) for Demographics. The groups are well matched for age and Leiter IQ.

Group	Age (months)	Leiter IQ	Vineland-II	ADOS	SCQ	Vestib
ASD	105.67 (25.09)	101.20 (24.73)	81.20 (14.14)	10.53 (5.50)	20.60 (7.43)	42.93
TD	108.60 (23.31)	104.47 (15.51)	99.80 (14.64)	N/A	1.80 (1.78)	50.67

** vestibular subsection only from the Sensory Profile Caregiver Questionnaire*

Table 2. Summary of Saccade T-test Comparisons for Gain and Latency. Horizontal saccades had average latency ~50 sec longer for ASD than for TD children (for whom average latency was similar to that reported in the literature).

Measure	Direction	Group	*n*	*M*	SD	*t*	Sig.
Gain	Horizontal	ASD	15	0.86	0.11		
		TD	17	0.91	0.60	-1.62	0.120
	Vertical	ASD	15	1.15	0.38		
		TD	17	1.10	0.24	0.45	0.657
Latency	**Horizontal**	ASD	15	0.26	0.06		
		TD	17	0.21	0.04	2.67	***0.012**
	Vertical	ASD	15	0.26	0.06		
		TD	17	0.24	0.62	0.85	0.405

**p < 0.05, statistically significant difference between groups*

Table 3. Summary of Smooth Pursuit T-test Comparisons for Gain (no significant differences)

Target condition (measure)	Group	*n*	*M*	SD	*T*	Sig.
Horizontal 0.1 Hz	ASD	15	1.02	0.18		
	TD	17	1.00	0.04	0.38	0.704
Horizontal 0.5 Hz	ASD	15	0.97	0.23		
	TD	17	1.02	0.09	-0.67	0.511
Vertical 0.1 Hz	ASD	14	1.07	0.29		
	TD	17	1.04	0.14	0.42	0.680
Vertical 0.5 Hz	ASD	15	0.85	0.23		
	TD	17	0.94	0.13	-1.32	0.200

Table 4. Summary of Smooth Pursuit T-test Comparisons for Phase. ASD children show vertical phase lags.

Target condition	Group	*n*	*M*	SD	*t*	**Sig.**
Horizontal 0.1 Hz	ASD	14	4.25	9.53		
	TD	17	0.85	1.42	1.37	0.193
Horizontal 0.5 Hz	ASD	15	6.71	8.11		
	TD	17	4.32	4.22	1.06	0.296
Vertical 0.1 Hz	ASD	15	10.03	9.94		
	TD	17	3.37	5.86	2.32	**0.028**
Vertical 0.5 Hz	ASD	15	14.93	13.71		
	TD	17	6.30	8.00	2.34	**0.029**

Table 5. Summary of Gaze Evoked Nystagmus Tests (no differences between groups)

Target condition	Group	*M*	SD	*t*	Sig.
Off_Right	ASD	0.83	4.14		
	TD	1.20	1.78	-0.25	0.804
Off_Left	ASD	-0.04	1.11		
	TD	-0.32	1.13	0.67	0.508
On_Right	ASD	1.15	2.83		
	TD	-0.96	2.49	1.94	0.065
On_Left	ASD	0.39	1.36		
	TD	0.37	1.99	0.04	0.972
Off_Up	ASD	1.00	2.06		
	TD	1.08	3.25	-0.07	0.949
Off_Down	ASD	0.24	1.60		
	TD	-0.13	1.51	0.59	0.565
On_Up	ASD	0.65	3.16		
	TD	0.08	3.16	0.44	0.668
On_Down	ASD	0.50	1.79		
	TD	0.59	2.32	-0.11	0.915

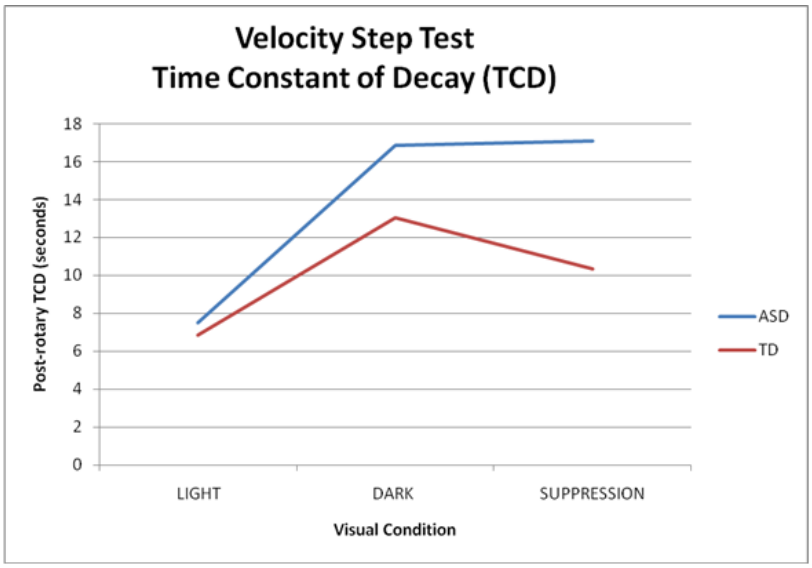

Figure 1 compares time constants for decay of post-rotary nystagmus for ASD and TD groups under three conditions of visual stimulation. The Light condition indicates that the surroundings were illuminated and fully visible; Dark indicates that the participant was wearing goggles with opaque occluders so that the surroundings were not visible and the visual field was dark; Suppression indicates that small red fixation spots were illuminated inside the dark opaque goggles. The presence of fixation spots had the expected effect of suppressing post-rotary nystagmus in TD children (i.e., reduced time constants for nystagmus decay relative to the Dark condition) but had a markedly reduced suppressing effect on the duration of post-rotary nystagmus in ASD children.

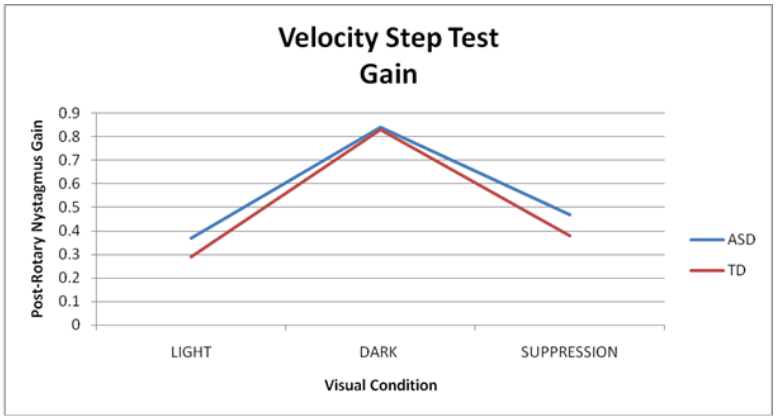

Figure 2 shows that post-rotary nystagmus gains (amplitudes of the eye movements) were affected similarly in ASD and TD groups by the same three visual conditions described for Figure 1.

KEY RESEARCH ACCOMPLISHMENTS

• Equipment needed to study vestibulo-ocular reflexes is fully operational with data integrated into databases for comparing VOR findings to corresponding neuropsychological test scores and demiographics.

• Twenty-five non-ASD typically developing child participants were tested fully with this equipment to date, and the data obtained were of good quality and within norms.

• Sixteen ASD children have been tested fully to date, and the data obtained were of good quality and of great interest. Findings reported in year 3 (Carson, T., Wilkes, B., Patel, K. and White, K. (2013) Rotary Vestibulo-Ocular Reflex Suppression and Slow Phase Velocity Differences in Autism Presented at the annual meeting of the Association for Research in Otolaryngology, Feb. 16, 2013, Baltimore, MD) include:

Abnormal VOR suppression in ASD. Presenting a visible fixation stimulus leads to rapid suppression of VOR eye movements in non-ASD typically developing children but this suppression is significantly reduced in ASD children.

Omissions of some fast-phase components during VOR in ASD. In non-ASD typically developing children the VOR nystagmus beats are always comprised of coupled slow-phase and fast phase pairs of repetitive eye movements. In many ASD children, some VOR nystagmus beats show a slow phase, a stationary pause rather than the fast phase movement toward primary position, then another slow phase in the same direction as the preceding one. During such beats the slow phase is “de-coupled” from the fast phase.

REPORTABLE OUTCOMES

Carson, T., Wilkes, B., Patel, K. and White, K. (2013) Rotary Vestibulo-Ocular Reflex Suppression and Slow Phase Velocity Differences in Autism. Poster presented at the annual meeting of the Association for Research in Otolaryngology, Feb. 16, 2013, Baltimore, MD.

CONCLUSION

Year 1 was delayed significantly by equipment failure and the acquisition and installation of new equipment. The new equipment was functional as of Year 1 Month 12, at which time we had obtained representative VOR data from 10 young adult participants. During year 2 we completed testing of 19 child participants, only one of whom has ASD diagnosis. By the end of year 3 we have completed testing of 41 child participants, 16 with ASD and 25 non-ASD controls.

Recruitment of children with ASD is continuing past year 3 during a no-cost extension year. We have already completed testing of an additional ASD child, and more children with ASD are in process of being scheduled for testing. Recruitment will cease by July 2013 because Jill Welsh, the lab member trained to administer ADOS diagnostic tests, will be leaving for a clinical internship at Johns Hopkins. Bradley Wilkes will carry out data analyses during the period from July – December 2013, at which time it is anticipated that he will be awarded his Master's degree. It is anticipated that Jill Welsh and Tana Bleser may each be awarded PhD degrees during the no-cost extension year.

It is premature to evaluate this project's findings as a potential medical product until data analyses are completed during the no-cost extension year. However, preliminary partial analyses have already shown two characteristics of VOR eye movements that differ reliably between ASD and non-ASD children, namely VOR suppression via fixation stimuli, and de-coupled slow and fast phases of some VOR nystagmus beats.

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